The Examiner has rejected Claims 13-16 and 18-32 under 35 USC §112, first paragraph, as allegedly containing subject matter not described in the specification in such a way as to reasonably convey, to one of ordinary skill in the art, that Applicants had possession of the claimed invention at the time the application was filed. Applicants respectfully submit that the Examiner's allegation is incorrect. The Examiner is splitting hairs in stating that the claims do not reflect what is taught by the specification. Examples 4,5,6 and 12 all show the 80%/20% composition. The same and other Examples show formulations where the alcohol (or ketone) is present at less than 80% and in combination with DMSO present at greater than 20%. Applicants respectfully submit that it is improper and unreasonable for the Examiner to expect that "concentrations 0.001% to 79.99%" would have to be taught to allow a statement of "less than" 80% to be allowed.

As far as the cell types that someone skilled in the art would recognize as falling under the present invention, Examples 4,5,6 and 7 show RNA stability for a bacteria (prokaryote), a yeast (a cell wall containing eukaryote), and a protozoan (eukaryote), and Example 13 shows structural integrity for additional human and insect cells. Finally, Example 11, with the most preferred embodiment, demonstrates RNA stability in whole blood, serum and plasma, with plasma being the most challenging from an RNase point of view.

In the present Official Action, these arguments about the cells were rejected by the Examiner as allegedly not persuasive. Applicants respectfully submit that it is unreasonable to expect that examples with different cells showing nucleic acid preservation, and other examples with different cells showing structure preservation would not be enough to allow a claim to structure and nucleic acid. Based on the Example 11 data, Applicants respectfully submit one skilled in the art would accept that the inventors were in possession of the present invention. Accordingly, withdrawal of the present rejection under Section 112 is respectfully requested.

The Examiner has rejected Claims 21 and 23 under 35 USC §112, second paragraph, as allegedly being indefinite for failing to distinctly claim the subject matter of the present invention. Applicants have amended Claims 21 and 23 by changing

the dependency in these claims from cancelled Claim 17 to pending Claim 16. Applicants thus respectfully request withdrawal of this rejection.

Claims 13-16,18 and 21-32 have been rejected under 35 USC §103(a) as allegedly rendered unpatentable by Gee et al., Williams et al., Connelly et al., Tometsko, Evinger-Hodges et al. and Bresser et al. The prior cited art does not teach or suggest the claimed invention. Applicants note that the above rejection does not refer to Claims 19 and 20, but these claims are referred to by the Examiner on Page 9 of the Official Action. Applicants respectfully submit that even if these claims are part of the present rejection (which at this point in time, they are not), the prior art does not teach the claimed subject matter therein either.

The claimed invention is directed to a method for stabilizing the structure and nucleic acids of at least one cell in a sample, wherein said method comprises:

(a) adding to a vessel containing the sample, a composition comprising:

(i) a first substance having a concentration effective for precipitating or denaturing proteins, comprising at least one alcohol or ketone whose concentration is less than 80% of the total composition; and

(ii) a second facilitator substance having a concentration effective for aiding in the infusion of the first substance into said at least one cell whose concentration is greater than 20% of the total composition, wherein the combined concentration of said first and second substances is equal to 100% of said composition;

(b) contacting said at least one cell in said sample with said composition;

(c) incubating said sample with said composition for an effective period of time and at an effective temperature;

(d) obtaining said at least one cell with stabilized structure and nucleic acids in said sample.

The Examiner alleges that Gee et al. teaches (in column 30, lines 54-56) structure and nucleic acid preservation. In actuality, Gee et al. teach a series of steps for visualizing cells and cell components by fixation and for allowing bulky dye

constructions to enter the cell. Gee et al. do not teach nucleic acid preservation by cell fixation, or a method for preserving the structure and nucleic acid of a cell, and certainly do not teach a vessel containing the sample for the preservation process. In Example 127, for example, there is a mention of mRNA, but one is not taught how to preserve it for further study. What are "standard methods?" Is only one molecule preserved? One of ordinary skill in the art is left with no teaching from Gee et al. of how to preserve the mRNA. Thus, Gee et al. do not teach or suggest the claimed invention.

The Examiner cites Williams et al. (page 1558, third paragraph), but in this reference there is only mention of ethanol at concentrations of 50%, 70% and 95%. There is no mention of DMSO in Williams et al. If one takes Example 3 in the present application, it is clear that many so-called fixatives, including 95% methanol, do not work to adequately "preserve" nucleic acids, contrasting with the Examiner's statement and use of Williams et al. as support.

Connelly et al. disclose a composition which is entirely different from the composition utilized in the method of the claimed invention. The fixative composition referred to by the Examiner in Connelly et al is alcohol-free (see column 4, line 6). This is in direct contrast to the present invention, which teaches a method for stabilizing the structure and nucleic acids of at least one cell in a sample, which furthermore utilizes a composition containing alcohol. Connelly et al. also use a substance to increase the permeability of a cell membrane and/or facilitate the transport of components across cell membranes. However this substance, as taught by Connelly et al., appears to be a detergent (column 8, lines 5-59), or a PEG-like material (column 7, lines 46-53), and although DMSO is mentioned (for example, column 17, lines 66-67), the taught concentration in Connelly et al. is 14%, well under the concentration of greater than 20% claimed in the present invention. Also the methods disclosed in Connelly et al. are different from the claimed method in the present invention. The importance of alcohol-free compositions in Connelly et al. is illustrated in Example 1, and specifically in column 17, lines 18-21.

In Tometsko, the DMSO treatment is separate and prior to "fixation." In addition, it is in the presence of an additional component, 1% BSA. Also, it is poured off prior to fixation. It is, in fact, a cell wash, not a preservative. Obviously, the method illustrated in Tometsko is different from the method claimed in the present invention. Based on the teachings of Tometsko, it is clear that the DMSO concentration in the fixation step (after pouring off the bulk of the DMSO) is very low, if significant at all. This bears no relationship to the present invention, which combines an alcohol and DMSO (no BSA, not separate steps) in one reagent. In addition, Tometsko argues that one of the most important steps to perform the cell fixation is at ultra low temperatures (see column 6, lines 60-61). Tometsko clearly teaches away from the present invention.

With respect to Evinger-Hodges, this reference discloses a method for in situ hybridization, not for preserving cells and/or structures and nucleic acids. In the Evinger-Hodges reference, cells are first deposited on a solid surface for processing. This is not the case in the present invention. The Examiner alleges that this reference also uses a "facilitator." That facilitator appears to be part of the hybridization composition (see page 14, lines 32-35). The composition in the Evinger-Hodges reference is clearly different in the present invention in that there is no deposition on a solid surface; no separate steps of "fixation" and then pore formation; no hybridization composition; and different cell preservation reagent compositions.

The final cited reference is Bresser et al. This is a method for in situ hybridization or immunocytology. Bresser et al. does mention DMSO as a possible permeability enhancer, but only in a larger group of possible compounds (column 1, lines 36-40). The enhancer is part of the "assay solution" (column 2, lines 19-40) and not part of the cell preservation formulation. In any case, the method taught in Bresser et al. is different than that claimed in the present invention. In addition, Bresser et al. teach rather low concentrations of the active components (2-20% DMSO, 2-20% alcohol, etc. see column 2, lines 50-65). As was previously pointed out to the Examiner, in previous Amendments, Bresser et al. clearly teaches away

from the claimed invention; and provides no additional teachings in combination with other cited references.

The Examiner has alleged that it would have been obvious to combine the teachings of Gee et al. with some or all of these additional references to achieve the claimed invention. However Gee et al. provides no effective teachings that could even be combined with information in the other cited references. What is missing from Gee et al. is the method of preserving nucleic acids. It is not at all clear to one of ordinary skill that the Gee et al. approach would be effective for nucleic acids. Thus, one might use Williams et al. as an approach for nucleic acids. However, Williams et al. is specific for M. tb genomic DNA and uses ethanol. By reviewing Example 3 of the present application, one can see that that approach was tested and did not work. Thus, Williams et al. teaches away from the present invention. Therefore, attempting to combine the cited references does not achieve the claimed invention.

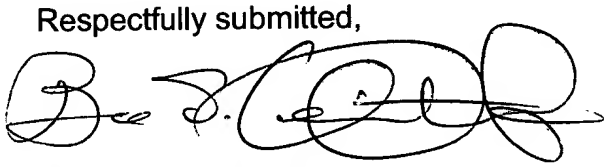
It is well established that obviousness cannot be established by combining the teachings of the prior art to produce the claimed invention, absent some teaching, suggestion or incentive supporting the combination, Carella v Starlight Archery, 804 F.2d 135, 231 U.S.P.Q. 644 (Fed. Cir. 1986). In determining obviousness, the inquiry is not whether each element existed in the prior art, but whether the prior art made obvious the invention as a whole for which patentability is claimed. Hartness International, Inc. v Simplimatic Engineering Co., 189 F.2d 1100, 2 U.S.P.Q. 2d 1826 (Fed. Cir. 1987). Furthermore, the Examiner cannot use hindsight reconstruction to pick and choose among isolated disclosures in the prior art to depreciate the claimed invention, In re Fine, 837 F.2d 1071, 5 U.S.P.Q. 2d 1596 (Fed. Cir. 1988), which the Examiner has clearly done in order to reject the claims under 35 U.S.C.§103.

Applicants have carefully studied the cited art as applied by the Examiner to reject the present claims and respectfully assert that the cited art does not render the teachings of the present invention obvious to one of ordinary skill in the art. Therefore, it is believed that the rejections of the claims under 35 U.S.C.§103 is improper, and withdrawal of these rejections is respectfully requested.

In view of the above Amendments and Remarks, Applicants believe that the present invention is in condition for allowance, which action is earnestly solicited.

Attached hereto is a marked-up version of the changes made to the claims by the current amendment. The attached page is captioned "**Version with markings to show changes made.**"

Respectfully submitted,



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VERSION WITH MARKINGS TO SHOW CHANGES MADE

IN THE CLAIMS

Claims 21 and 23 have been amended as follows:

21. (Amended). The method of Claim [17] 16 wherein said first substance is methanol and said second substance is dimethyl sulfoxide.

23. (Amended). The method of Claim [17] 16 wherein said first substance is ethanol and said second substance is dimethyl sulfoxide.